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Comparative studies on the antifungal activity of triadimefon, triadimenol, fenarimol, nuarimol, imazalil and fluotrimazole in vitro

Vergleichende Untersuchungen über die fungitoxische Wirkung von Triadimefon, Triadimenol, Fenarimol, Nuarimol, Imazalil and Fluotrimazol in vitro

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Summary

The antifungal activity of various fungicides towards some fungi was determined by the ED_{50} and MIC (minimal inhibitory concentration) values for mycelial growth, sporidia multiplication and spore germination. On the basis of their ED_{50} values members of the Oomycetes and Zygomycetes were moderately or marginally sensitive towards triadimefon, triadimenol, fenarimol, nuarimol and imazalil. All five chemicals proved to be toxic or highly toxic against several fungal species belonging to the classes of Ascomycetes (as for instance *Erysiphe graminis* f. sp. *hordei*, *Sphaerotheca fuliginea*, *Gaeumannomyces graminis*, *Sordaria fimicola*), Deuteromycetes (e. g. *Septoria nodorum*, *Colletotrichum trifolii*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Pleiochaeta setosa*, *Drechslera graminea* (except triadimefon), *Bipolaris sorokiniana* (except triadimefon), *Fusarium culmorum*, *Verticillium albo-atrum*, *V. dahliae*) and Basidiomycetes (e. g. *Rhizoctonia solani*, *Ustilago avenae*). The antifungal spectrum of fluotrimazole appeared to be almost entirely restricted to powdery mildews but the compound showed a certain degree of activity towards smut fungi. While in some fungi relatively small differences between the ED_{50} and MIC values were found in other species great variations between both values were evident. The compounds only poorly affected conidial germination in sensitive species (e. g. *Cladosporium cucumerinum*) whereas the hyphal development was strongly suppressed. Germ tubes of treated spores were abnormally swollen and malformed. No close relationship between the sensitivity towards the compounds tested and the taxonomic position of the fungal species could be established. There was no definite correlation between the in vitro activity of the compounds and their effectiveness in vivo.

Key words: systemic fungicides; triadimefon; triadimenol; fenarimol; nuarimol; imazalil; fluotrimazole; antifungal activity; mycelial growth; spore germination; germ tube growth; sporidia multiplication

Zusammenfassung

Zur Prüfung der fungitoxischen Wirkung von verschiedenen Fungiziden gegenüber einigen Pilzarten wurden die ED_{50}- und MHK (minimale Hemmkonzentration)-Werte

für das Myzelwachstum, Sporidienvermehrung und Sporenkeimung bestimmt. Auf der Grundlage der ED_{60}-Werte zeigten die zur Gruppe der Oomyceten und Zygomyceten gehörenden Pilzarten eine mäßige bis schwache Empfindlichkeit gegenüber Triadimefon, Triadimenol, Fenarimol, Nuarimol und Imazalil. Alle fünf Verbindungen erwiesen sich als toxisch gegenüber mehreren Pilzarten, die verschiedenen Klassen angehören: Ascomycetes (wie z. B. *Erysiphe graminis* f. sp. *hordei, Sphaerotheca fuliginea, Gaeumannomyces graminis, Sordaria fimicola*), Deuteromycetes (z. B. *Septoria nodorum, Colletotrichum trifolii, Botrytis cinerea, Cladosporium cucumerinum, Pleiochaeta setosa, Drechslera graminea* (mit Ausnahme von Triadimefon), *Bipolaris sorkiniana* (außer Triadimefon), *Fusarium culmorum, Verticillium albo-atrum, V. dahliae*) und Basidiomycetes (z. B. *Rhizoctonia solani, Ustilago avenae*). Das antimykotische Wirkungsspektrum von Fluotrimazol scheint sich nahezu gänzlich auf die Echten Mehltaupilze zu beschränken; eine gewisse Wirksamkeit zeigte die Substanz noch gegenüber Brandpilzen. Während bei einigen Pilzen verhältnismäßig geringe Unterschiede zwischen den ED_{60}- und MHK-Werten gefunden wurden, traten bei anderen Arten große Unterschiede zwischen beid Werten auf. Die Substanzen beeinflußten die Konidienkeimung bei empfindlichen Arten (z. B. *Cladosporium cucumerinum*) nur schwach, dagegen wurde die Keimschlauchentwicklung stark unterdrückt. Es konnte keine enge Beziehung zwischen der Empfindlichkeit gegenüber den Fungiziden und der taxonomischen Stellung der verschiedenen Pilzarten festgestellt werden. Auch wurde keine eindeutige Korrelation zwischen der in vitro-Aktivität der Substanzen und ihrer Wirksamkeit in vivo nachgewiesen.

Stichwörter: systemische Fungizide; Triadimefon; Triadimenol; Fenarimol; Nuarimol; Imazalil; Fluotrimazol; fungitoxische Wirkung; Myzelwachstum; Sporenkeimung; Keimschlauchentwicklung; Sporidienvermehrung

1 Introduction

Several new antifungal compounds belonging chemically to the classes of triazole, pyrimidine and imidazole derivatives have recently been developed. Triadimefon [MEB 6447; 1-(4-chlorophenoxy)-3,3-dimethyl-(1,2,4-triazol-1-yl)-2-butanone] is recommended for foliar application to control powdery mildews, rusts, *Typhula incarnata* and *Rhynchosporium secalis* (Frohberger 1973, 1975; Kaspers et al. 1975; Ebenebe and Fehrmann 1974; Kampe 1975; Buchenauer 1976; Scheinpflug et al. 1977). Triadimenol [KWG 0519; 1-(4-chlorophenoxy)-3,3-dimethyl-(1,2,4-triazol-1-2-butanol] is structurally related to triadimefon and has been developed for seed treatment to combat various important plant pathogens like smuts *(Ustilago hordei, U. nuda, U. nigra, U. avenae, Urocystis occulta)*, bunts *(Tilletia caries, T. foetida)*, seedborne *Septoria nodorum, T. incarnata, R. secalis, Pyrenophora avenae* and *Cochliobolus sativus*. Following seed treatment (30–37,5 g a.i./100 kg seed) the compound protects cereal shoots for about two months against mildew infection and also offers a significant protection against rust infections of cereal seedlings. After seed treatment the compound is insufficiently effective against *Fusarium* spp. (e. g. *Calonectria nivalis*), *Pyrenophora graminea* and *Tilletia controversa* (Frohberger 1977).

Fenarimol [EL-222; α-(2-chlorophenyl)-α-(4-chlorophenyl)-5-pyrimidine methanol] in field trials effectively controlled *Venturia inaequalis* on leaves and fruits and its eradicative activity against this disease was demonstrated in greenhouse tests. The compound was found to show a high level of effectiveness against powdery mildews, e. g. *Erysiphe cichoracearum* on zinnias and *E. graminis* f. sp. *hordei* on barley (Brown et al. 1975). Following seed treatment nuarimol (EL-228; α-(2-chlorophenyl)-α-(4-

fluorophenyl)-5-pyrimidine methanol) controlled several seed borne diseases of barley and other cereals such as *C. nivalis, S. nodorum, P. avenae, Ustilago* spp., *Tilletia caries* and *Typhula incarnata*. While nuarimol showed a high effectiveness against *P. graminea* in spring barley its activity towards this disease in winter barley was insufficient. Following both seed and foliar treatment nuarimol proved to be highly active against barley mildew (CASANOVA et al. 1977).

Imazalil {1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole} is reported to control *Penicillium* molds and *Geotrichum candidum* on citrus (LAVILLE 1973; HARDING 1976), *Mycosphaerella fijiensis* var. *musicola* on bananas (MELIN et al. 1976), *P. graminea* and *C. sativus* on barley as well as *P. avenae* on oats (BARTLETT and BALLARD 1975).

While the toxicants mentioned above display systemic properties, fluotrimazole [BUE 0620; 1-(3-trifluoromethyltriphenyl)-1,2,4-triazole] is predominantly active as a protective fungicide and the antifungal spectrum of this compound is almost exclusively limited to powdery mildews (GREWE and BÜCHEL 1973; JEFFREY et al. 1975).

The present investigation reports on the in vitro toxicity of these compounds towards a certain number of fungal species belonging to different taxonomic groups. Preliminary results of this work have already been published (BUCHENAUER 1977a).

2 Material and methods

2.1 Antifungal agents

For these investigations the following compounds were used: Triadimefon (98,6 %), triadimenol (93,7 %) and fluotrimazole (98,0 %) (Bayer AG, Leverkusen), fenarimol (95,0 %) and nuarimol (96,2 %) (Eli Lilly, Bad Homburg) as well as imazalil-nitrate (95 %, corresponding 82 % basic equivalent) (Janssen Pharmaceutica, Beerse, Belgium).

2.2 Fungitoxicity test

2.2.1 Mycelial growth

The fungal species belonging to different taxonomic groups were usually maintained on malt extract agar. The effect of the chemicals on mycelial radial growth was determined by dissolving the fungicides at various concentrations in acetone, and suspending aliquots in malt agar at 50°C to give the required series of concentrations. The final acetone concentration in both fungicide-containing and control samples was 1 %. The medium also contained streptomycin-sulfate (50 µg/ml) to suppress bacterial growth except when fungi belonging to the Oomycetes were tested. Petri dishes containing 10 ml of the agar medium were inoculated by placing 6 mm agar disks grown with the fungus upside down on the agar surface. Plates were incubated at 23°C and radial growth was measured after definite incubation periods.

2.2.2 Spore germination

Powdery mildews: The in vitro effectiveness of the chemicals towards the powdery mildew of barley *(Erysiphe graminis* f. sp. *hordei)* and cucumber *(Sphaerotheca fuliginea)* was determined by the germination rate and germ tube growth of conidia. A method similar to that described by DE WAARD (1971) was applied. Cellulose membranes (25 µm thick) were placed upon a modified Czapek-Dox-agar medium in Petri dishes and young conidia of both powdery mildews were dusted onto the membranes. To avoid condensation of water on the membranes the covers of the dishes were lined

with filter paper disks soaked with glycerol. Plates were kept upside down at room temperature (21°C ± 1°C) and after 48 h incubation the germination rate was determined.

Rust fungi and *Cladosporium cucumerinum:* In order to determine the toxicity of the chemicals on the germination of urediospores of *Puccinia recondita* f. sp. *tritici* and *Uromyces appendiculatus* the spores were previously washed with sterile distilled water containing 0.01 % of Tween 80 and then suspended in sterile distilled water (2×10^5 spores/ml). Conidia suspension of *C. cucumerinum* (4×10^5 conidia/ml) was obtained from young mycelium of the fungus grown on malt agar in Petri dishes. Equal volumes of the spore suspensions and of the fungicidal solutions or suspensions were mixed and two drops of the mixture were transferred onto glass slides. After definite incubation periods at 21°C ± 1°C and 100 % rel. humidity in the dark, germination tubes were stained with cotton blue in lactophenol and both germination rate and length of germ tubes were evaluated.

2.2.3 Sporidia multiplication

Sporidia of *Ustilago avenae* were grown in Erlenmeyer flasks (300 ml) containing 50 ml of a nutrient solution (BUCHENAUER 1977 b) at 27°C on a rotary shaker (100 rpm). The initial inoculum density varied from 2×10^7 to 2×10^9 sporidia/ml and the effect of the fungicides on the sporidia replication was determined by counting the cells in a hemacytometer after various incubation periods.

2.3 Determination of the toxicity

The antifungal activity of the fungicides was determined as ED_{50} (concentration inhibiting growth by 50 % compared with the control) and as MIC (minimal inhibiting concentration, that is the lowest concentration producing total inhibition).

According to their sensitivity (ED_{50} and MIC) towards the compounds examined the fungi were classified into the following groups:

—	>500 µg/ml (insensitive)
±	100–500 µg/ml (marginally sensitive)
+	50–100 µg/ml (slightly sensitive)
++	10– 50 µg/ml (moderately sensitive)
+++	2– 10 µg/ml (sensitive)
++++	< 2 µg/ml (very sensitive)
n. t.	not tested

3 Results

The comparative fungitoxic activity of triadimefon, triadimenol, fenarimol, nuarimol, imazalil and fluotrimazole towards a limited number of fungal species belonging to different taxonomic groups is shown in Tables 1 and 2. As far as the ED_{50} values are concerned the Oomycetes and Zygomycetes appeared to be moderately or marginally sensitive towards the compounds tested, with the exception of fluotrimazole. *Pythium irregulare* responded more sensitively to nuarimol than *Pythium ultimum*.

Fungal species examined within the Ascomycetes varied from very sensitive to marginally sensitive. *Gaeumannomyces graminis* and *Sordaria fimicola* were highly sensitive towards triadimefon, triadimenol, fenarimol, nuarimol and imazalil, the

MIC-values were also very low. While imazalil, nuarimol and fenarimol proved to be toxic to *Monilinia fructigena* triadimefon and triadimenol were less active.

The fungitoxic spectrum of fluotrimazole appeared to be confined to powdery mildews but the compound showed a certain degree of activity against smut fungi (Tables 1 and 2).

Within the Sphaeropsidales, *Septoria nodorum* was sensitive towards triadimenol and triadimefon and very sensitive to imazalil, fenarimol and nuarimol. A similar degree of sensitivity to triadimefon and triadimenol was detected for *Ascochyta phleina*, however the MIC-values were higher for *A. phleina* than for *S. nodorum*. While imazalil was highly toxic against *Pyrenochaeta lycopersici*, triadimefon and triadimenol were less active.

Among the Melanconiales the sensitivity of *Colletotrichum trifolii* towards the chemicals was rather high.

Great variations in the sensitivity of species tested within the Hyphomycetes were found. The toxicity of the chemicals towards species with blastosporic conidia formation was relatively high. Fenarimol, nuarimol, and imazalil proved to be toxic to very toxic towards fungi belonging to the group of Porosporae. Compared with triadimenol, triadimefon showed a remarkable inferior toxicity against these fungi, especially to *D. graminea* and *B. sorokiniana*.

Geotrichum candidum, a representative of the Arthrosporae, proved to be only slightly sensitive to the fungicides tested.

Fungal species examined within the Phialosporae also varied greatly in their sensitivity. This was even true for species belonging to the same genus, e. g. *F. moniliforme* and *F. culmorum* which were more sensitive than *F. oxysporum*. The sensitivity of *Scopulariopsis brevicaulis* (Annellosporae) and *Pseudocercosporella herpotrichoides* (Radulasporae) towards the various fungicides increased in the following order: triadimefon < triadimenol < fenarimol = nuarimol < imazalil.

The toxicity of the chemicals against sporidia of *Ustilago avenae* (at 2×10^7 sporidia/ml) which was measured by inhibition of sporidia multiplication was high. A distinct inoculum effect was observed with *U. avenae* sporidia and the toxicants tested. Increased sporidia density resulted in increased ED_{50} and MIC-values.

The effect of the fungicides on germination of conidia of powdery mildews and of urediospores of rust fungi is shown in Table 2. Although – as it will be pointed out later – the compounds tested generally proved to be less toxic to spore germination than to mycelium growth both mildew fungi *(Erysiphe graminis* f. sp. *hordei* and *Sphaerotheca fuliginea)* were sensitive to all six chemicals but compared with the ED_{60}-values for complete inhibition of conidia germination 30–40 times higher concentrations of the toxicants were needed. Elongation of germ tubes was affected slightly more than germination.

Germination of urediospores of both rust fungi was less influenced by the fungicides than conidial germination of the powdery mildews. Spores of *Puccinia recondita* f. sp. *tritici* were more sensitive towards triadimefon, triadimenol, fenarimol, nuarimol and imazalil than those of *Uromyces appendiculatus*. The chemicals inhibited growth of germ tubes more severely than spore germination. Fluotrimazole showed no or only a slight activity towards both rust fungi.

The effect of the compounds on conidial germination and germ tube growth of *Cladosporium cucumerinum* is presented in Table 3. While triadimefon, triadimenol, fenarimol, nuarimol and imazalil effectively inhibited mycelial growth of *C. cucumerinum* the compounds did not appreciably reduce the spore germination rate even at

Table 1. Antifungal spectrum of triadimefon, triadimenol, fenarimol, nuarimol, imazalil and fluotrimazole. The fungitoxic activities (ED_{50} and MIC-values) refer to inhibition of mycelium growth on malt agar (explanation of symbols see page 344)

Fungal species tested	Days	Triadimefon ED_{50}	Triadimefon MIC	Triadimenol ED_{50}	Triadimenol MIC	Fenarimol ED_{50}	Fenarimol MIC	Nuarimol ED_{50}	Nuarimol MIC	Imazalil ED_{50}	Imazalil MIC	Fluotrimazole ED_{50}	Fluotrimazole MIC
Oomycetes													
Pythium irregulare Buism.	2	++	±	++	+	++	±	+++	±	+	±	—	—
Pythium ultimum Trow	2	++	±	++	+	++	+	+	±	+	±	—	—
Phytphthora cryptogea Pethybr. & Laff.	5	+	±	n.t.	n.t.	n.t.	n.t.	±	—	+	±	n.t.	n.t.
Phytophthora nicotianae (Breda de Haan) Tucker	5	++	+	n.t.	n.t.	n.t.	n.t.	±	—	+	±	—	—
Zygomycetes													
Mucor hiemalis Wehmer	4	±	—	++	±	++	±	++	±	++	±	—	—
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind.	3	±	—	+	±	+	±	++	±	++	±	—	—
Ascomycetes													
Gaeumannomyces graminis (Sacc.) v. Arx & Oliver	9	++++	++	++++	++	++++	++	++++	+++	++++	+++	n.t.	n.t.
Monilinia fructigena (Pers.) Sacc.	5	+	—	++	+	+++	++	+++	++	+++	+++	—	—
Sordaria fimicola (Roberge) Ces. et de Not	4	+++	++	+++	++	++++	++	++++	++	+++	+++	—	—
Deuteromycetes													
Sphaeropsidales													
Septoria nodorum (Berk.) Berk	12	+++	++	+++	++	+++	++	+++	++	++++	++	—	—
Ascochyta phleina	5	+++	±	+++	±	n.t.	n.t.	n.t.	n.t.	+	+	n.t.	n.t.
Phoma glomerata (Corda) Wollenw. et Hochapfel	5	±	—	+	±	n.t.	n.t.	n.t.	n.t.	+	+	—	—
Pyrenochaeta lycopersici Schneid. et Gerl.	12	++	+	++	+	n.t.	n.t.	n.t.	n.t.	++++	+++	n.t.	n.t.

Melanconiales													
Colletotrichum trifolii Bain et Ess.	9	+++	±	+++	±	+++	+	+++	++	+++	++	—	—
Pestalotia paeoniae	6	+	—	++	±	+++	+	++	+	+	±	—	—
Moniliales (Hyphomycetes)													
Blastosporae													
Botrytis cinerea Pers. ex Fr.	5	++	+	+++	+	+++	+	++	+	++++	+++	—	—
Cladosporium cucumerinum Ellis & Arth.	9	++++	++	++++	+++	++++	+++	++++	+++	++++	+++	±	—
Pleiochaeta setosa (Kirchn.) Hughes	10	+++	++	+++	+++	++++	+++	++++	+++	++++	+++	n.t.	n.t.
Porosporae													
Alternaria alternata (Fr.) Keiss.	7	±	—	+	+	++++	+++	++++	+++	+++	++	—	—
Alternaria solani Sor.	7	±	—	++	±	++++	+++	++++	+++	+++	++	n.t.	n.t.
Drechslera graminea (Rabenh.) Shoem.	9	±	±	+++	+	+++	++	++++	++	++++	++++	n.t.	n.t.
Bipolaris sorokiniana (Sacc.) Shoem.	10	+	±	+++	±	++++	++	++++	++	++++	+++	—	—
Arthrosporae													
Geotrichum candidum Link	6	++	—	++	±	±	—	±	—	++	±	—	—
Phialosporae													
Aspergillus niger van Tiegh.	6	++	—	+	—	+++	++	+++	++	+++	++	n.t.	n.t.
Fusarium oxysporum Schlecht. et Fr.	5	++	—	++	—	++	±	+++	±	+++	++	n.t.	n.t.
Fusarium moniliforme Sheld	6	++++	—	++++	±	+++	++	++++	++	+++	+++	—	—
Fusarium culmorum (W. G. Sm.) Sacc.	6	+++	++	++++	++	+++	+	+++	+	++++	++	n.t.	n.t.
Penicillium cyclopium Westling	8	+	—	++	±	++	±	++	±	++	+	—	—
Thielaviopsis basicola (Berk. & Br.) Ferr.	10	+	—	++	+	+++	+	+++	±	++++	++++	n.t.	n.t.

Table 1. (Continued).

Fungal species tested	Days	Triadimefon		Triadimenol		Fenarimol		Nuarimol		Imazalil		Fluotrimazole	
		ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC
Verticillium albo-atrum Reinke & Berth.	10	+++	++	++++	+++	++++	++	+++	++	++++	+++	—	—
Verticillium dahliae Kleb.	10	+++	+	+++	++	+++	++	+++	++	++++	+++	—	—
Verticillium lecanii (Zimm.) Viegas	6	++	±	++++	++	++	±	++	±	++++	++	—	—
Anellosporae													
Scopulariopsis brevicaulis (Sacc.) Bain.	6	+	—	+	—	+++	±	+++	±	++++	+++	n.t.	n.t.
Radulasporae													
Pseudocercosporella herpotrichoides (Fron) Deighton	10	+	±	++	±	++++	++	++++	++	++++	+++	—	—
Mycelia sterilia *Rhizoctonia solani* Kühn	5	+++	±	+++	+	+++	++	+++	+++	++	+	n.t.	n.t.
Basidiomycetes													
Ustilago avenae (Pers.) Rostr.[1])	1	++++	++	++++	++	++++	++	++++	++	+++	++	++	+

[1]) Toxicity was determined by sporidia multiplication

Table 2. Effect of various fungicides on spore germination of *Erysiphe graminis* f. sp. *hordei*, *Sphaerotheca fuliginea*, *Puccinia recondita* f. sp. *tritici* and *Uromyces appendiculatus* (explanation of symbols see page 344)

Fungal species tested	Incubation time (h)	Triadimefon ED_{50}	Triadimefon MIC	Triadimenol ED_{50}	Triadimenol MIC	Fenarimol ED_{50}	Fenarimol MIC	Nuarimol ED_{50}	Nuarimol MIC	Imazalil ED_{50}	Imazalil MIC	Fluotrimazole ED_{50}	Fluotrimazole MIC
Ascomycetes													
Erysiphe graminis DC. f. sp. *hordei* Em. Marchal	48	+++	±	+++	±	+++	±	+++	+	+++	±	+++	±
Sphaerotheca fuliginea (Schlecht ex Fr.) Poll.	48	+++	±	+++	±	+++	±	+++	±	+++	±	+++	±
Basidiomycetes													
Puccinia recondita f. sp. *tritici* (Erikss.) C.O. Johnston	18	++	±	++	±	++	±	++	±	+++	++	±	—
Uromyces appendiculatus (Pers.) Lk.	16	+	—	+	—	++	—	+	—	n.t.	n.t.	—	—

Table 3. Effect of various fungicides on germination and germ tube growth of conidia of *Cladosporium cucumerinum* after an incubation period of 18 h at 21 ± 1°C

Compound	Conc. (µg/ml)	Germination (% of control)	Length of germ tube (% of control)
Triadimefon	62,5	95	46
Triadimefon	125	87	48
Triadimefon	250	85	40
Triadimenol	62,5	88	43
Triadimenol	125	85	42
Triadimenol	250	85	39
Fenarimol	62,5	88	43
Fenarimol	125	90	40
Fenarimol	250	84	36
Nuarimol	62,5	90	39
Nuarimol	125	83	34
Nuarimol	250	74	35
Imazalil	62,5	68	32
Imazalil	125	47	29
Imazalil	250	34	27
Fluotrimazole	62,5	97	92
Fluotrimazole	125	98	92
Fluotrimazole	250	98	77

the highest concentration applied. Imazalil proved to be somewhat more effective. On the other hand germ tube development was strongly suppressed by the compounds. Germ tubes of the treated conidia were abnormally thickened and deformed. Fluotrimazole affected neither germination nor germ tube development.

4 Discussion

Comparative investigations on the antifungal spectrum of triadimefon, triadimenol, fenarimol, nuarimol and imazalil revealed that on the basis of the ED_{50}-values all five chemicals were toxic to highly toxic towards numeral fungal species belonging to the class of Ascomycetes (e. g. *Erysiphe graminis* f. sp. *hordei, Sphaerotheca fuliginea, Gaeumannomyces graminis, Sordaria fimicola),* Deuteromycetes (e. g. *Septoria nodorum, Colletotrichum trifolii, Botrytis cinerea, Cladosporium cucumerinum, Pleiochaeta setosa, Fusarium culmorum, Verticillium albo-atrum, V. dahliae)* and Basidiomycetes *(Rhizoctonia solani, Ustilago avenae).* The antifungal spectrum of the compounds tested nearly corresponded to that of triforine (Fuchs and Drandarevski 1973; Drandarevski and Fuchs 1973).

No close relationship between toxicity of the chemicals and taxonomic classification of the fungi could be established. This was in contrast to studies on the antifungal spectrum of benomyl which revealed a close correlation between the in vitro toxicity and the taxonomic position of the fungi (Bollen and Fuchs 1970; Edgington et al. 1971). Even fungi within the same genus showed differential sensitivity to the compounds investigated. For instance *Fusarium oxysporum* showed a higher degree of tolerance than *F. culmorum* or *F. moniliforme* and among the *Verticillium* species tested *V. albo-atrum* was more sensitive than *V. lecanii.* In their studies on the antifungal spectrum of triforine Fuchs and Drandarevski (1973) and Drandarevski and

Fuchs (1973) obtained similar results; the authors found large variations in sensitivity towards triforine e. g. within the genera *Ascochyta*, *Aspergillus*, *Phoma* and *Verticillium*.

The imidazole derivatives miconazole – structurally related to imazalil – and clotrimazole – chemically similar to fluotrimazole – which according to their primary mode of action also belong to the group of ergosterol biosynthesis inhibitors (Van den Bossche et al. 1978; Buchenauer 1978) possess a wide spectrum of activity against most fungi and yeasts of medical interest. Both compounds have been reported to show a variable toxicity to closely related fungi in vitro. Differential activity of miconazole has been found towards some species and strains of *Aspergillus* (Symoens 1977) and also *Candida* species markedly varied in their susceptibility to this agent; for instance. *C. parapsilosis* and *C. pseudotropicalis* were highly susceptible whereas *C. pelliculosa*, *C. intermedia* and *C. tropicalis* needed about 1000 fold higher concentrations for their complete inhibition. Furthermore, within the species *C. albicans* the isolates differed in their susceptibility to miconazole, the MIC-values required varying between 0,5 and 32 µg/ml (Bannatyne and Cheung 1978). Similar results have been reported by Van Cutsem and Thienpont (1972) and Shadomy et al. (1977). The MIC-values for clotrimazole towards different strains of *C. albicans*, *Microsporum canis*, *Trichophyton soudanense* and *Nocardia brasiliensis* varied greatly (Waitz et al. 1971; Plembel and Bartmann 1972; Kunicki 1974).

Unexpectedly, the fungicides exerted a slight toxicity towards some representatives of the Oomycetes (e. g. *Pythium irregulare);* it is assumed that these toxicants interfere in ergosterol biosynthesis and it is generally accepted that members of the Oomycetes do not contain sterols in their membranes (Hendrix 1964, 1966, 1970; Elliott et al. 1964; Haskins et al. 1964). Our findings however are not surprising if one takes into consideration the microbial spectrum of activity of miconazole and clotrimazole. Besides their antifungal activity, clotrimazole and miconazole showed a high degree of in vitro toxicity against gram-positive bacteria (e. g. strains of *Staphylococcus* and *Streptococcus)* (Plembel and Bartmann 1972; Van Cutsem and Thienpont 1972; Schär et al. 1976) and it is known that bacteria do not have sterols as architectural components in their membranes. Thus these results imply that apart from their inhibition of ergosterol biosynthesis, these toxicants may have a secondary mode of action in fungi.

Differential effectiveness among the various compounds examined became evident, for instance the outstanding toxicity of imazalil towards *Drechslera graminea* and *Bipolaris sorokiniana*. Triadimenol proved to be superior to triadimefon in its toxicity. In contrast to the other compounds the toxic spectrum of fluotrimazole appeared to be predominately focused on powdery mildews.

As the data in Tables 1 and 2 show the degree of toxicity of a compound towards a fungus is only incompletely described by its ED_{50}-values. In some fungi relatively small differences between the ED_{50} and the MIC-values were found but in other species great differences between both values were evident. Therefore the in vitro determination of both values for a fungal species may also be of advantage for predicting the effectiveness of a toxicant in controlling this fungal pathogen in vivo since it is assumed that within various fungal species causing a similar type of disease those are controlled possibly more effectively which show small differences between the ED_{50} and MIC-values towards a toxicant in vitro.

The results further suggested that no close correlation exists between the in vitro toxicity of the compounds tested and their in vivo effectiveness in controlling the fungal pathogens in plants. For example, although the compounds – which are systemically active – showed a noticeable toxicity in vitro towards *V. albo-atrum* they failed

to control *Verticillium* wilt of cotton and tomato following root treatment in the greenhouse. Likewise, no significant reduction of eye spot disease caused by *Pseudocercosporella herpotrichoides* was obtained in the field after spray application (unpublished results). While the seed borne *Septoria* may be effectively eliminated by seed treatment the effectiveness in controlling the glume blotch following foliar treatment is insufficient (FROHBERGER 1973, 1977).

On the other hand the toxicants are much more active in vivo against powdery mildews than would have been predicted by their in vitro activity. This may be due to the fact that the compounds generally affect spore germination less severely than mycelium growth as the results with *Cladosporium cucumerinum* have indicated. Studies on the site of interference of the chemicals during the infection process of *Erysiphe graminis* f. sp. *hordei* revealed that the formation of haustoria was inhibited by the compounds at substantial lower concentrations than conidia germination (BUCHENAUER 1976). Similar effects have been reported by FUCHS and DRANDAREVSKI (1973) and DRANDAREWSKI and FUCHS (1973) comparing the in vitro toxicity of triforine with i performance in vivo. In contrast, a close correlation between the in vitro activity of benomyl and its effectiveness in vivo in controlling plant diseases could be established (BOLLEN and FUCHS 1970).

The results revealed a close dependence of the toxicity of the compounds upon the density of *Ustilago avenae* sporidia in the liquid nutrient medium. In studies on the determination of the MIC-values of clotrimazole in *Candida albicans*, PLEMBEL and BARTMANN (1972) and WAITZ et al. (1971) also found that MIC-values increased with the increase in inoculum density or incubation time. PLEMBEL and BARTMANN (1972) suggested that the inoculum effect may be ascribed to a partial and reversible binding of clotrimazole to the cell surfaces.

Analogous morphological alterations of germ tubes have been found with triforine and triarimol in *Cladosporium cucumerinum* (SHERALD et al. 1973), with Denmert in *Monilinia fructigena* (KATO et al. 1974) and with fenarimol in sensitive fungi (BROWN and HALL 1978).

Factors which may possibly account for the specific action of fluotrimazole are e. g. limited uptake or rapid breakdown by insensitive fungal species.

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